IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Appl. No. : **10/767,329**

Applicants : **Sean D. Monahan et al.**

Filed : **01/29/2004**

Art Unit : **1615**

Examiner : **Barhan, Bethany P.**

Docket No. : **Mirus.041.01**

For: **Protein and Peptide Delivery to Mammalian Cells In Vitro**

Commissioner of Patents
PO Box 1450
Alexandria, VA 22313-1450

DECLARATION UNDER 37 C.F.R. §1.132

Dear Sir:

I, Dr. Vladimir Trubetskoy, hereby declare as follows:

1. I have a Doctorate in biochemistry from the University of USSR Academy of Medical Sciences (Moscow, Russia).

2. I am familiar with the above captioned application.

3. I am familiar with lipids and detergents used in chemical and biological sciences.

4. The Action dated July 2, 2007 for the above captioned application indicates that the Examiner is interpreting phosphatidylcholine molecules to be detergents. However, this interpretation is not in agreement with accepted scientific principles.

   Surfactants are defined in the art and in the above captioned application as amphiphilic (containing both hydrophobic and hydrophilic parts) molecules which reduce the surface tension of water or between two immiscible liquid phases (water and oil, for example).

   In lipid biophysics the difference between detergents and lipids is determined by the type of aggregates these molecules form in water. Detergents form micelles in water and are thus generally considered to be soluble in water. Classical lipids may form bilayers or inverted hexagonal (H_{II}) phases in aqueous solution and are thus generally considered to be soluble in water. For example Kumar (PNAS 88, 444 (1991)) teaches that the ability of an amphipathic

molecule to be classified as detergent or classical lipid is dependent on packing parameter S, which can be calculated. According to this theory detergents (such as lysophosphatidyl-choline) possess S < 1/2. Bilayer-forming lipids (such as phosphatidylcholine) have ½<S<1. H_{II}-forming lipids have S>1. Another example of this definition can be found in Ai and Caffrey (Biophysical Journal Volume 79 July 2000 394–405), where they discuss the complementary shapes of detergent and H_{II}-forming lipid as indication to form bilayer.

The definition of detergents as surfactants capable of forming micelles in aqueous solutions is widespread in lipid biophysics. The issue whether phosphatidylcholines (PC) can be classified as detergents in this sense depends on two parameters: 1) the number of acyl chains of the PC molecule; and 2) the number of carbon atoms in each chain. LysoPC, which by definition contains only one acyl chain is always a detergent no matter how many carbon atoms it contains. However, PC, which by definition contains two acyl chains can form micelles (i.e. be classified as detergents) only if the number of atoms in both acyls contain less than 8 carbon atoms (see Kumar 1991). However, PCs isolated from natural sources have two chains each with greater than 8 acyl carbons (typically 16-22 carbons, see "Fatty acid content of tissue-derived phosphatydilcholine" from Avanti Polar Lipids).

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

10/2/07

Dr. Vladimir Trubetskoy Date